Synthesis of carbon-11-labeled 5-HT$_6$R antagonists as new candidate PET radioligands for imaging of Alzheimer's disease

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Abstract—Carbon-11-labeled serotonin (5-hydroxytryptamine) 6 receptor (5-HT$_6$R) antagonists, 1-[(2-bromophenyl)sulfonyl]-5-[^{11}C]methoxy-3-[(4-methyl-1-piperazinyl)methyl]-1*H*-indole (*O*-[^{11}C]**2a**) and 1-[(2-bromophenyl)sulfonyl]-5-methoxy-3-[(4-[^{11}C]methyl-1-piperazinyl)methyl]-1*H*-indole (*N*-[^{11}C]**2a**), 5-[^{11}C]methoxy-3-((4-methylpiperazin-1-yl)methyl)-1-(phenylsulfonyl)-1*H*-indole (*O*-[^{11}C]**2b**) and 5-methoxy-3-((4-[^{11}C]methylpiperazin-1-yl)methyl)-1-(phenylsulfonyl)-1*H*-indole (*N*-[^{11}C]**2b**), 1-((4-isopropylphenyl)sulfonyl)-5-[^{11}C]methoxy-3-((4-methylpiperazin-1-yl)methyl)-1*H*-indole (*O*-[^{11}C]**2c**) and 1-((4-isopropylphenyl)sulfonyl)-5-methoxy-3-((4-[^{11}C]methylpiperazin-1-yl)methyl)-1*H*-indole (*N*-[^{11}C]**2c**), 1-((4-fluorophenyl)sulfonyl)-5-[^{11}C]methoxy-3-((4-methylpiperazin-1-yl)methyl)-1*H*-indole (*O*-[^{11}C]**2d**) and 1-((4-fluorophenyl)sulfonyl)-5-methoxy-3-((4-[^{11}C]methylpiperazin-1-yl)methyl)-1*H*-indole (*N*-[^{11}C]**2d**), were prepared from their *O*- or *N*-desmethylated precursors with [^{11}C]CH_3OTf through *O*- or *N*-[^{11}C]methylation and isolated by HPLC combined with SPE in 40-50% radiochemical yield, based on [^{11}C]CO_2 and decay corrected to end of bombardment (EOB). The radiochemical purity was >99%, and the molar activity (MA) at EOB was 370-740 GBq/μmol with a total synthesis time of ~40-minutes from EOB.

Keywords: Serotonin (5-hydroxytryptamine) 6 receptor (5-HT$_6$R); Carbon-11-labeled 5-HT$_6$R antagonists; Radiosynthesis; Positron emission tomography (PET); Alzheimer's disease (AD).

Alzheimer's disease (AD) is the most common form of dementia and affects close to 50 million people worldwide in 2017.[1] The disease is divided into four stages, including predementia, early dementia, moderate AD and advanced AD.[2] The cause for most AD cases is still unknown, and there are several competing hypotheses like genetic, cholinergic hypothesis, amyloid hypothesis, and tau hypothesis, trying to explain it.[3] AD might be treated by symptomatic treatments and disease-modifying therapies such as neuroprotective and neurorestorative therapies, however, none effective strategy is approved for preventing, curing and slowing the progress of AD.[4] The current available medications can only be used to treat the cognitive problems of AD, focused on acetylcholinesterase inhibitors (AChEIs) including tacrine, rivastigmine, galantamine, and donepezil, as well as a *N*-methyl-$_D$-aspartate (NMDA) receptor antagonist memantine.[5] The benefit from these approved cognitive enhancing drugs for AD is small, and novel alternate therapy for treating cognitive disorders is eagerly needed.[6] Since the clinical trial of the disease-modifying therapies in AD is an extremely complex process with very high failure rate, the researchers have turned their focus to symptomatic

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treatment, and serotonin (5-hydroxytryptamine) 6 receptor (5-HT_6R) has become a promising alternative target.[7] 5-HT_6R is a subtype of 5-HT receptor, and it is a G protein-coupled receptor (GPCR). 5-HT_6R antagonists have been hypothesized to improve cognition, learning, and memory.[8]

To discover more effective treatments, reliable diagnostic tools are really needed. Neuroimaging of AD becomes one of the most active as well as most challenging areas in neuroscience.[9] Advanced biomedical imaging technique positron emission tomography (PET) is a promising modality for AD, and significant advances have occurred in this field of molecular imaging.[10] The development of PET imaging probes for *in vivo* detection of Alzheimer's brains is critical for early and accurate diagnosis and for the successful discovery of AD therapies.[11] Previous PET AD imaging agent development is based on cholinergic hypothesis, amyloid hypothesis, and tau hypothesis. The representative PET tracers in clinical evaluations such as carbon-11-labeled AChEIs [^{11}C]AMP ([^{11}C]MP4A), [^{11}C]PMP ([^{11}C]MP4P), and [^{11}C]Donepezil;[12-14] β-amyloid plaques (Aβ) tracers [^{11}C]PIB and [^{18}F]Amyvid ([^{18}F]AV-45);[15,16] and tau tracers [^{11}C]PBB3 and [^{18}F]T807 ([^{18}F]AV-1451)[17,18] are listed in Figure 1. The success and limitations of Aβ imaging and tau imaging have spurred efforts worldwide to develop new selective PET tracers for different imaging targets. Our efforts toward the development of PET agents for AD diagnosis have been ongoing quite some time, and a series of enzyme- or receptor-based PET agents have been developed in this laboratory. In our previous work, we have targeted the enzyme glycogen synthase kinase-3 (GSK-3) and developed carbon-11-labeled GSK-3 inhibitors[19,20] as PET AD imaging agents (Figure 1). In this ongoing study, we target 5-HT_6R, which is a novel and attractive molecular target for treatment and PET imaging of AD.[8] Several 5-HT_6R PET tracers such as [^{18}F]12ST05, [^{11}C]GSK215083 and [^{11}C]SB399885, as shown in Figure 2, have been previously developed, however, preclinical and clinical evaluation indicated these radioligands have significant drawbacks like no specific binding, high non-specific binding, poor brain entry and inconsistent brain uptake compared to known 5-HT_6R distribution.[21-23] Thus an ideal 5-HT_6R radioligand that can be used in the clinical setting to study 5-HT_6R expression levels in AD remains to be discovered. Recently a novel series of 3-(piperazinylmethyl) indole derivatives have been developed as 5-HT_6R antagonists for potential treatment of AD, and the representative compounds, 1-[(2-bromophenyl)sulfonyl]-5-methoxy-3-[(4-methyl-1-piperazinyl)methyl]-1*H*-indole (**2a**), 5-methoxy-3-((4-methylpiperazin-1-yl)methyl)-1-(phenylsulfonyl)-1*H*-indole (**2b**), 1-((4-isopropylphenyl)sulfonyl)-5-methoxy-3-((4-methylpiperazin-1-yl)methyl)-1*H*-indole (**2c**) and 1-((4-fluorophenyl)sulfonyl)-5-methoxy-3-((4-methylpiperazin-1-yl)methyl)-1*H*-indole (**2d**), exhibited high binding affinity to human 5-HT_6R with K_i value 2.0, 4.3, 1.6 and 5.0 nM, respectively, and high selectivity over 100 target sites.[24] These compounds possesses the combination of favorable binding affinity and selectivity to 5-HT_6R, and *O*- and *N*-methyl positions amenable to labeling with carbon-11, therefore, their carbon-11-labeled radioligands are expected to have high specific binding. Here, we report the synthesis of carbon-11-labeled 5-HT_6R antagonists labeled at oxygen or nitrogen positions *O*-[^{11}C]**2a-d** and *N*-[^{11}C]**2a-d** (Figure 2) as new potential PET radioligands for imaging of AD.

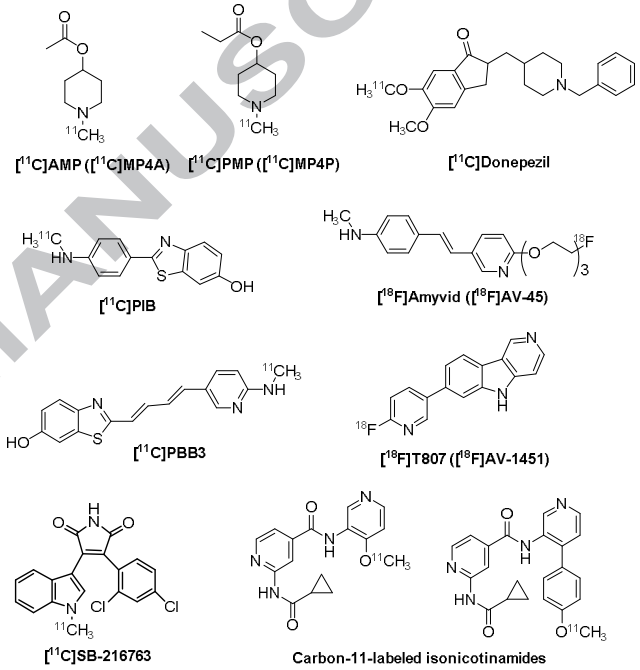


Figure 1. PET radiotracers for imaging of AD.

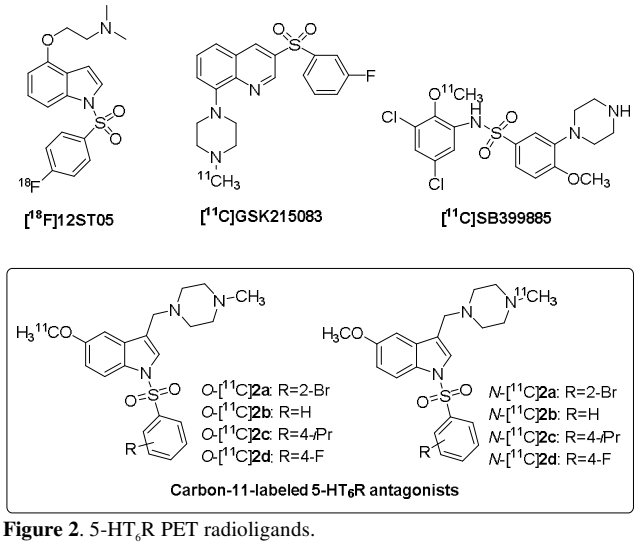
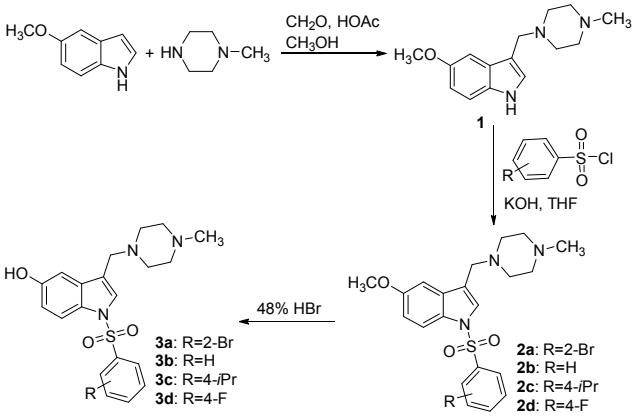
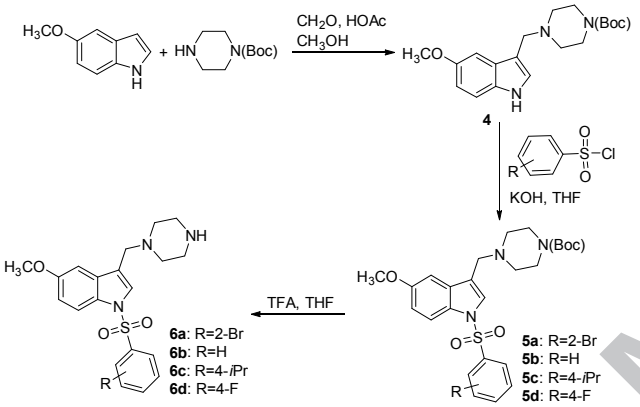


Figure 2. 5-HT_6R PET radioligands.

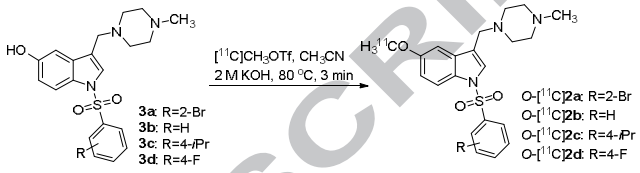


Scheme 1. Synthesis of reference standards **2a-d** and *O*-desmethylated precursors **3a-d**.

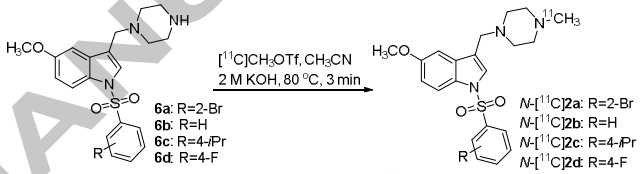


Scheme 2. Synthesis of *N*-desmethylated precursors **6a-d**.

The reference standards **2** (**2a**: R = 2-Br; **2b**: R = H; **2c**: R = 4-*i*Pr; **2d**: R = 4-F), their *O*-desmethylated precursors **3** (**3a**: R = 2-Br; **3b**: R = H; **3c**: R = 4-*i*Pr; **3d**: R = 4-F) and *N*-desmethylated precursors **6** (**6a**: R = 2-Br; **6b**: R = H; **6c**: R = 4-*i*Pr; **6d**: R = 4-F) were synthesized as depicted in Scheme 1 and Scheme 2 according to the reported procedure with modifications.[24-29] The main modifications included the use of different reagents, reactions and methods to synthesize new compounds *O*- and *N*-desmethylated precursors. 5-Methoxy-3-((4-methylpiperazin-1-yl) methyl)-1*H*-indole (**1**) and *tert*-butyl 4-((5-methoxy-1*H*-indol-3-yl)methyl)piperazine-1-carboxylate (**4**) were achieved by well-known Mannich reaction from commercially available 5-methoxy-1*H*-indole and 1-methylpiperazine or *tert*-butyl piperazine-1-carboxylate in 92% and 91% yield, respectively. Reference standards **2** (**2a**: R = 2-Br; **2b**: R = H; **2c**: R = 4-*i*Pr; **2d**: R = 4-F) was prepared from commercially procured aryl sulfonyl or substituted aryl sulfonyl by condensation with compound **1** in the presence of ground KOH in THF with 76-94% yield. Compounds **2** (**2a**: R = 2-Br; **2b**: R = H; **2c**: R = 4-*i*Pr; **2d**: R = 4-F) were treated with 48% HBr to provide the corresponding *O*-desmethylated precursors **3** (**3a**: R = 2-Br; **3b**: R = H; **3c**: R = 4-*i*Pr; **3d**: R = 4-F) in 88-94% yield. Compounds **5** (**5a**: R = 2-Br; **5b**: R = H; **5c**: R = 4-*i*Pr; **5d**: R = 4-F) were prepared from commercially procured aryl sulfonyl or substituted aryl sulfonyl by condensation with compound **4** in the presence of ground KOH in THF with 90-96% yield. The deprotecting reaction of compounds **5** (**5a**: R = 2-Br; **5b**: R = H; **5c**: R = 4-*i*Pr; **5d**: R = 4-F) with trifluoroacetic acid (TFA) in THF gave *N*-desmethylated precursors **6** (**6a**: R = 2-Br; **6b**: R = H; **6c**: R = 4-*i*Pr; **6d**: R = 4-F) in 91-96% yield.



Scheme 3. Synthesis of target tracers (*O*-[^{11}C]**2a-d**) labeled at oxygen position.



Scheme 4. Synthesis of target tracers (*N*-[^{11}C]**2a-d**) labeled at nitrogen position.

Synthesis of the target tracer (*O*-[^{11}C]**2a-d** and *N*-[^{11}C]**2a-d**) labeled at oxygen or nitrogen positions is shown in Scheme 3 and Scheme 4. *O*-Desmethylated precursors **3a-d** and *N*-desmethylated precursors **6a-d** underwent *O*- or *N*-[^{11}C]methylation[30,31] using the reactive [^{11}C]methylating agent [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[32,33] in acetonitrile at 80 °C under basic conditions (2 M KOH). The product was isolated by semi-preparative reverse-phase (RP) high performance liquid chromatography (HPLC) with a C-18 column, and then concentrated by solid-phase extraction (SPE)[34,35] with a disposable C-18 Light Sep-Pak cartridge to produce the corresponding pure radiolabeled compound *O*-[^{11}C]**2a-d** or *N*-[^{11}C]**2a-d** in 40-50% radiochemical yield, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2.

The radiosynthesis includes three stages: 1) labeling reaction; 2) purification; and 3) formulation. We employed more reactive [^{11}C]CH_3OTf, instead of commonly used [^{11}C]methyl iodide ([^{11}C]CH_3I),[36] in *O*- or *N*-[^{11}C]methylation to improve radiochemical yield of *O*-[^{11}C]**2a-d** or *N*-[^{11}C]**2a-d** from 30-40% to 40-50%. We used an Eckert & Ziegler Modular Lab C-11 Methyl Iodide/Triflate module to produce [^{11}C]methylating agent either [^{11}C]CH_3OTf or [^{11}C]CH_3I ([^{11}C]CH_3Br passed through a NaI column). The direct comparison

between [^{11}C]CH_3OTf and [^{11}C]CH_3I confirmed the result. The labeling reaction was conducted using a V-vial method. Addition of aqueous $NaHCO_3$ to quench the radiolabeling reaction and to dilute the radiolabeling mixture prior to the injection onto the semi-preparative HPLC column for purification gave better separation of *O*-[^{11}C]**2a-d** or *N*-[^{11}C]**2a-d** from its *O*-desmethylated precursors **3a-d** or *N*-desmethylated precursors **6a-d**. We used Sep-Pak trap/release method instead of rotatory evaporation for formulation to improve the chemical purity of radiolabeled products *O*-[^{11}C]**2a-d** or *N*-[^{11}C]**2a-d**. In addition, a C18 Light Sep-Pak to replace a C18 Plus Sep-Pak allowed final product formulation with ≤5% ethanol.[37] Overall, it took ~40 min for synthesis, purification and dose formulation.

The radiosynthesis was performed in a home-built automated multi-purpose [^{11}C]-radiosynthesis module.[38-40] This radiosynthesis module facilitated the overall design of the reaction, purification and reformulation capabilities in a fashion suitable for adaptation to preparation of human doses. In addition, the module is designed to allow in-process measurement of [^{11}C]-tracer molar activity (MA, GBq/μmol at EOB) using a radiation detector with a UV detector at the outlet of the HPLC-portion of the system. In the HPLC chromatogram, peak analysis of the chromatographic data utilized PeakSimple software (SRI Instruments, Las Vegas, NV). Immediately following elution of the product peak, the chromatographic data are exported to PeakSimple readable files, and the area of the radioactivity peak is converted to GBq - mCi at EOB by comparison to a reference calibration curve previously constructed using the same detector, loop column, mobile phase and flow rate. The mass peak from the UV chromatogram (without decay correction) is similarly compared to a standard curve made at the same UV wavelength, mobile phase and flow rate. Simple division of the total EOB radioactivity peak (in GBq - mCi) by the total mass peak (in nmoles) gives specific activity at EOB in GBq - Ci/μmol. For the reported syntheses, product MA was in a range of 370-740 GBq/μmol at EOB. The factors that affect the EOB MA significantly to lead to such a wide range have been discussed in our previous works.[41] The general methods to increase SA have been described as well, and the SA of our [^{11}C]-tracers is significantly improved.[41] The 'wide range' of MA we reported is for the same [^{11}C]-tracer produced in different days, because very different [^{11}C]-target and [^{11}C]-radiosynthesis unit situations would make MA in a wide range. For a [^{11}C]-tracer produced in the same day, the MA of the same tracer in different production runs will be in a small range, because [^{11}C]-target and [^{11}C]-radiosynthesis unit would not be much different in the same day. Likewise, the methods to minimize such wide range of MA from practice perspective have been provided in our previous works.[42] At the end of synthesis (EOS), the MA of [^{11}C]-tracer was determined again by analytical RP HPLC, calculated, decay corrected to EOB, and based on [^{11}C]CO_2, which was in agreement with the 'on line' determined value. In this work, semi-preparative HPLC was used for purification, thus the MA of [^{11}C]-tracer was assessed by both semi-preparative HPLC (during synthesis) and analytical HPLC (EOS).

Chemical purity and radiochemical purity were determined by analytical HPLC.[43] The chemical purity of the precursor and reference standard was >95%. The radiochemical purity of the target tracer was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of the target tracer was >95% determined by reversed-phase HPLC through UV flow detector.

The octanol-water partition coefficient (commonly expressed as Log P) is an important physical parameter directly correlated with the biological activities of a wide variety of organic compounds.[42] Log P provides an assessment of lipophilicity that often correlates with a compound's ability to penetrate the blood brain barrier (BBB). We obtained Log P and calculated Log P (CLog P) values of carbon-11-labeled 5-HT_6R antagonists *O*-[^{11}C]**2a-d** and *N*-[^{11}C]**2a-d** (Figure 2) in comparison with [^{11}C]PIB, [^{18}F]Amyvid, [^{11}C]PBB3 and [^{18}F]T807 (Figure 1) from ChemDraw Professional 15.1 (ChemOffice) as listed in Table 1. Log P data of *O*-[^{11}C]**2a-d** and *N*-[^{11}C]**2a-d** (2.59 - 3.83) are similar to those of [^{11}C]PIB, [^{18}F]Amyvid, [^{11}C]PBB3 and [^{18}F]T807 (2.25 – 4.09), which are PET AD imaging agents in clinical evaluation. These data suggest the *O*-[^{11}C]**2a-d** and *N*-[^{11}C]**2a-d** have appropriate lipophilicity for brain uptake.

Table 1. Log P and CLog P values of carbon-11-labeled 5-HT_6R antagonists **2a-d** in comparison with [^{11}C]PIB, [^{18}F]Amyvid, [^{11}C]PBB3 and [^{18}F]T807.

Compound	Log P	CLog P
***O*- or *N*-[^{11}C]2a**	3.42	4.92
***O*- or *N*-[^{11}C]2b**	2.59	4.05
***O*- or *N*-[^{11}C]2c**	3.83	5.48
***O*- or *N*-[^{11}C]2d**	2.75	4.20
[^{11}C]PIB	3.41	3.99
[^{18}F]Amyvid	3.16	3.91
[^{11}C]PBB3	4.09	4.05
[^{18}F]T807	2.25	3.18

The stability of the labeled tracers *O*-[^{11}C]**2a-d** and *N*-[^{11}C]**2a-d** was evaluated by analytical HPLC from EOS up to 3 h, one injection of the tracer solution in EtOH/saline onto HPLC column per hour. The HPLC

chromatograms showed *O*-[^{11}C]**2a-d** and *N*-[^{11}C]**2a-d** were stable without decomposition.

The experimental details and characterization data for compounds **1-6** and for the tracers *O*-[^{11}C]**2a-d** and *N*-[^{11}C]**2a-d** are given.[44]

In summary, synthetic routes with moderate to high yields have been developed to produce the reference standard **2a-d**, *O*-desmethylated precursor **3a-d**, *N*-desmethylated precursor **6a-d**, and target tracer *O*-[^{11}C]**2a-d** and *N*-[^{11}C]**2a-d**. The radiosynthesis employed [^{11}C]CH_3OTf for *O*- or *N*-[^{11}C]methylation at *O*- or *N*-position of 3-(piperazinylmethyl) indole desmethylated precursor, followed by product purification and isolation using a semi-preparative RP HPLC combined with SPE. *O*-[^{11}C]**2a-d** and *N*-[^{11}C]**2a-d** were obtained in high radiochemical yield, radiochemical purity and chemical purity, with a reasonable short overall synthesis time, and high molar activity. This will facilitate studies to evaluate carbon-11-labeled 5-HT_6R antagonists *O*-[^{11}C]**2a-d** and *N*-[^{11}C]**2a-d** as new candidate PET radioligands for imaging of AD.

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44. *(a). General:* All commercial reagents and solvents were purchased from Sigma-Aldrich and Fisher Scientific, and used without further purification. [^{11}C]CH_3OTf was prepared according to a literature procedure.[33] Melting points were determined on WRR apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker Avance II 600 MHz NMR Fourier transform spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to an internal standard tetramethylsilane (TMS, δ 0.0), and coupling constants (J) are reported in hertz (Hz). Liquid chromatography-mass spectra (LC-MS) analysis was performed on AB Sciex 4000Q Trap instrument, consisting of an 1100 series HPLC connected to a diode array detector and a 1946D mass spectrometer configured for positive-ion/negative-ion electrospray ionization (ESI). The high resolution mass spectra (HRMS) were obtained using a Waters/Micromass LCT Classic spectrometer. Chromatographic solvent proportions are indicated as volume: volume ratio. Thin-layer chromatography (TLC) was run using HS silica gel GF254 uniplates (5 × 10 cm^2). Plates were visualized under UV light. Normal phase flash column chromatography was carried out on Combiflash Rf 150 silica gel 60 (300-400 mesh) with a forced flow of the indicated solvent system in the proportions described below. All moisture- and air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Analytical RP HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6 × 250 mm, a gradient mobile phase (40-80%) CH_3CN/3 mM $HCOONH_4$, flow rate 1.6 mL/min; UV (254 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative RP HPLC column was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 10 × 250 mm; 57% CH_3CN:43% 0.1 M $NaHCO_3$ mobile phase; 5, 4, 7, and 5 mL/min flow rate for *O*-[^{11}C]**2a** and *N*-[^{11}C]**2a**, *O*-[^{11}C]**2b** and *N*-[^{11}C]**2b**, *O*-[^{11}C]**2c** and *N*-[^{11}C]**2c**, *O*-[^{11}C]**2d** and *N*-[^{11}C]**2d**, respectively; UV (254 nm) and γ-ray (PIN diode) flow detectors. C18 Light Sep-Pak cartridges were obtained from Waters Corporation (Milford, MA). Sterile Millex-FG 0.2 μm filter units were obtained from Millipore Corporation (Bedford, MA).
*(b). 5-Methoxy-3-[(4-methyl-1-piperazinyl)methyl]-1H-indole (**1**):* The formaldehyde solution (20 mL, 30% w/v, 0.2 mol) was added to the stirred mixture of 1-methylpiperazine (15.0 g, 0.15 mol) and acetic acid (6.0 g, 0.1 mol) in water (20 mL) at 10 ºC. The reaction mixture was stirred at 10 ºC for 15 min, it was warmed to room temperature (RT) and stirred for another 2 h. After the thick syrup was formed, it was added slowly to a stirred solution of 5-methoxy-1*H*-indole (20 g, 0.13 mol) in MeOH (125 mL) at 5 ºC. The reaction mixture was warmed and stirred at RT for another 3 h. The mixture was poured into ice water, the pH was adjusted to 10-12 with aqueous 5 N NaOH solution, and extracted with EtOAc. The combined organic layer was washed with water, brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under vacuum. The crude product was purified by silica gel column chromatography with CH_2Cl_2/MeOH (100:1 to 10:1) as eluent to afford **1** as a white solid (32.5 g, 92%), mp 79.5-80.4 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.55 (s, 1H), 7.15 (d, J = 2.4 Hz, 1H), 7.13 (d, J = 8.8 Hz, 1H), 6.95 (d, J = 1.9 Hz, 1H), 6.81 (dd, J = 2.4, 8.8 Hz, 1H), 3.86 (s, 3H), 3.71 (s, 2H), 2.48 (s, 8H), 2.30 (s, 3H). LC-MS (ESI, *m/z*): Calcd for $C_{15}H_{22}N_3O$ ([M+H]$^+$) 260.2, found: 260.2.
*(c). 1-[(2-Bromophenyl)sulfonyl]-5-methoxy-3-[(4-methyl-1-piperazinyl)methyl]-1H-indole (**2a**):* To a stirred mixture of finely ground KOH solid (0.93 g, 16.6 mmol) and **1** (2.0 g, 7.7 mmol) in THF (8.0 mL), 2-bromobenzenesulphonyl chloride (2.6 g, 10.0 mmol) in THF (5.0 mL) was added at 15 ºC. After the reaction mixture was stirred at RT for 3 h, the mixture was poured into ice water and the pH was adjusted to 9.5-10 with aqueous 2 N KOH solution. The mixture was extracted with EtOAc. The combined organic layer was washed with water, brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under vacuum. The crude product was purified by silica gel column chromatography with CH_2Cl_2/MeOH (200:1 to 20:1) as eluent to afford **2a** as a white solid (3.3 g, 90%), mp 127.7-128.8 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.04 (dd, J = 1.5, 8.0 Hz, 1H), 7.64 (t, J = 5.7 Hz, 2H), 7.54 (d, J = 9.0 Hz, 1H),

7.43 (t, *J* = 7.6 Hz, 1H), 7.36 (dd, *J* = 1.5, 7.6 Hz, 1H), 7.19 (d, *J* = 2.4 Hz, 1H), 6.84 (dd, *J* = 2.4, 8.9 Hz, 1H), 3.62 (s, 2H), 3.83 (s, 3H), 2.32-2.50 (br, 8H), 2.29 (s, 3H). LC-MS (ESI, *m/z*): Calcd for $C_{21}H_{25}BrN_3O_3S$ ($[M+H]^+$) 479.4, found: 479.9. Compounds **2b**, **2c**, and **2d** were prepared with the same procedure according to **2a**, and the data were listed as below. *5-Methoxy-3-((4-methylpiperazin-1-yl)methyl)-1-(phenylsulfonyl)-1H-indole (**2b**):* White solid (2.8 g, 90%), mp 125.1-125.4 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.87 (d, *J* = 9.0 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 2H), 7.44 (t, *J* = 7.4 Hz, 2H), 7.35 (t, *J* = 7.6 Hz, 2H), 7.14 (d, *J* = 2.0 Hz, 1H), 6.91 (dd, *J* = 2.0, 9.0 Hz, 1H), 3.79 (s, 3H), 3.55 (s, 2H,), 2.45 (br, 8H), 2.26 (s, 3H). LC-MS (ESI, *m/z*): Calcd for $C_{21}H_{26}N_3O_3S$ ($[M+H]^+$) 400.2, found: 400.0. *1-((4-Isopropylphenyl)sulfonyl)-5-methoxy-3-((4-methylpiperazin-1-yl)methyl)-1H-indole (**2c**):* White solid (2.6 g, 76%), mp 126.6-128.8 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.88 (d, *J* = 9.0 Hz, 1H), 7.74 (d, *J* = 8.4 Hz, 2H), 7.44 (s, 1H), 7.21 (d, *J* = 8.4 Hz, 2H), 7.16 (d, *J* = 2.1 Hz, 1H), 6.92 (dd, *J* = 2.1, 9.0 Hz, 1H), 3.79 (s, 3H), 3.57 (s, 2H), 2.86-2.81 (m, 1H), 2.46 (br, 8H), 2.26 (s, 3H), 1.13 (d, *J* = 7.0 Hz, 6H). LC-MS (ESI, *m/z*): Calcd for $C_{24}H_{32}N_3O_3S$ ($[M+H]^+$) 442.2, found: 442.0. *1-((4-Fluorophenyl)sulfonyl)-5-methoxy-3-((4-methylpiperazin-1-yl)methyl)-1H-indole (**2d**):* White solid (3.0 g, 94%), mp 130.6-134.0 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.85 (t, *J* = 8.8 Hz, 3H), 7.40 (s, 1H), 7.11 (d, *J* = 2.0 Hz, 1H), 7.08 (t, *J* = 8.5 Hz, 2H), 6.93 (dd, *J* = 2.0, 9.0 Hz, 1H), 3.83 (s, 3H), 3.60 (s, 2H), 2.59 (br, 8H), 2.42 (s, 3H). LC-MS (ESI, *m/z*): Calcd for $C_{21}H_{25}FN_3O_3S$ ($[M+H]^+$) 418.2, found: 417.9.

*(d). 1-((2-Bromophenyl)sulfonyl)-3-((4-methylpiperazin-1-yl)methyl)-1H-indol-5-ol (**3a**):* To a solution of HBr (7 mL, 48% wt), **2a** (2.0 g, 4.8 mmol) was added at RT under stirring, the resulting mixture was heated to 80 ºC and reaction continued for 12 h. The reaction mixture was poured into water (40 mL) after cooled to 25 ºC, and then extracted with $CHCl_3$ (3 × 40 mL) after the pH was adjusted to 10 with ammonia solution (17%). The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under vacuum. The crude product was purified by silica gel column chromatography with $CHCl_3$/CH_3OH (100:10) as eluent to afford **3a** as a pale yellow solid (1.8 g, 93%), mp 212.0-212.8 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.03 (d, *J* = 8.0 Hz, 1H), 7.59 (d, *J* = 5.0 Hz, 2H), 7.42 (dd, *J* = 7.7, 8.8 Hz, 2H), 7.34 (t, *J* = 7.7 Hz, 1H), 7.07 (s, 1H), 6.77 (d, *J* = 8.8 Hz, 1H), 3.56 (s, 2H), 2.69-2.62 (m, 8H), 2.39 (s, 3H). HRMS (ESI, *m/z*): Calcd for $C_{20}H_{23}BrN_3O_3S$ ($[M+H]^+$) 464.0643, found: 464.0633. Compounds **3b**, **3c**, and **3d** were prepared with the same procedure according to **3a**, and the data were listed as below. *3-((4-Methylpiperazin-1-yl)methyl)-1-(phenylsulfonyl)-1H-indol-5-ol (**3b**):* White solid (1.5 g, 94%), mp 211.3-212.8 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.86 (d, *J* = 7.5 Hz, 2H), 7.67 (d, *J* = 8.9 Hz, 1H), 7.63 (t, *J* = 7.5 Hz, 2H), 7.61 (br, 1H), 7.55 (t, *J* = 8.0 Hz, 2H), 7.08 (d, *J* = 2.1 Hz, 1H), 6.85 (dd, *J* = 2.1, 8.9 Hz, 1H), 4.23 (s, 2H), 3.47 (br, 8H), 2.60 (s, 3H). HRMS (ESI, *m/z*): Calcd for $C_{20}H_{24}N_3O_3S$ ($[M+H]^+$) 386.1538, found: 386.1538. *1-((4-Isopropylphenyl)sulfonyl)-3-((4-methylpiperazin-1-yl)methyl)-1H-indol-5-ol (**3c**):* White solid (1.6 g, 94%), mp 211.4-212.8 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.79 (d, *J* = 8.8 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 2H), 7.40 (s, 1H), 7.22 (d, *J* = 8.4 Hz, 2H), 6.96 (d, *J* = 2.0 Hz, 1H), 6.78 (dd, *J* = 2.0, 8.8 Hz, 1H), 3.51 (s, 2H), 2.88-2.83 (m, 1H), 2.49 (s, 8H), 2.26 (s, 3H), 1.16 (d, *J* = 6.9 Hz, 6H). HRMS (ESI, *m/z*): Calcd for $C_{23}H_{30}N_3O_3S$ ($[M+H]^+$) 428.2008, found: 428.2043. *1-((4-Fluorophenyl)sulfonyl)-3-((4-methylpiperazin-1-yl)methyl)-1H-indol-5-ol (**3d**):* Pale yellow solid (1.5 g, 88%), mp 210.8-212.8 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.97 (dd, *J* = 4.9, 8.8 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.56 (s, 1H), 7.41 (t, *J* = 8.8 Hz, 2H), 6.99 (d, *J* = 2.3 Hz, 1H), 6.81 (dd, *J* = 2.3, 8.8 Hz, 1H), 3.51 (s, 2H), 2.39 (s, 8H), 2.28 (s, 3H). HRMS (ESI, *m/z*): Calcd for $C_{20}H_{23}FN_3O_3S$ ($[M+H]^+$) 404.1444, found: 404.1419.

*(e). tert-Butyl 4-((5-methoxy-1H-indol-3-yl)methyl)piperazine-1-carboxylate (**4**):* The formaldehyde solution (1.60 mL, 30% w/v, 0.02 mol) was added to the stirred mixture of *tert*-butyl piperazine-1-carboxylate (1.50 g, 0.015 mol) and acetic acid (0.62 g, 0.01 mol) in water (1.6 mL) at 10 ºC. The reaction mixture was stirred at 10 ºC for 15 min, it was warmed to RT and stirred for another 2 h. After the thick syrup was formed, it was added slowly to a stirred solution of 5-methoxy-1*H*-indole (2.0 g, 0.013 mol) in methanol (13 mL) at 5 ºC. The reaction mixture was warmed to RT and stirred for another 3 h. The reaction mixture was poured into ice water, and the pH was adjusted to 10-12 with aqueous 5 N NaOH solution and extracted with EtOAC. The combined organic layers were washed with water, brine, dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated under vacuum. The crude product was purified by silica gel column chromatography with CH_2Cl_2/MeOH (100:1 to 10:1) as eluent to afford **4** as a white solid (4.1 g, 91%), mp 132.8-134.0 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.31 (d, *J* = 8.8 Hz, 1H), 7.09 (d, *J* = 2.2 Hz, 1H), 6.86 (dd, *J* = 2.2, 8.8 Hz, 1H), 6.83 (s, 1H), 3.83 (s, 3H), 3.54 (s, 2H), 3.29 (t, *J* = 4.5 Hz, 4H), 2.36 (t, *J* = 4.5 Hz, 4H), 1.42 (s, 9H). LC-MS (ESI, *m/z*): Calcd for $C_{19}H_{27}N_3O_3$($[M+Na]^+$) 368.2, found: 368.0.

*(f). tert-Butyl 4-((1-((2-bromophenyl)sulfonyl)-5-methoxy-1H-indol-3-yl)methyl)piperazine-1-carboxylate (**5a**):* To a stirred mixture of finely ground KOH solid (0.93 g, 16.6 mmol) and **4** (2.7 g, 7.7 mmol) in THF (8.0 mL), 2-bromobenzenesulphonyl chloride (2.6 g, 10.0 mmol) in THF (5.0 mL) was added at 15 ºC. After the reaction mixture was stirred

at RT for 3 h, the mixture was poured into ice water and the PH was adjusted to 9.5-10 with aqueous 2 N KOH solution. The mixture was extracted with EtOAC. The combined organic layer was washed with water, brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated under vacuum. The crude product was purified by silica gel column chromatography with CH_2Cl_2/MeOH (200:1 to 20:1) as eluent to afford **5a** as a white solid (3.9g, 89.7%), mp 136.9-138.7 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.09 (d, J = 6.4 Hz, 1H), 7.66 (d, J = 7.7 Hz, 2H), 7.54 (d, J = 9.0 Hz, 1H), 7.46 (t, J = 7.7 Hz, 1H), 7.39 (t, J = 7.4 Hz, 1H), 7.20 (s, 1H), 6.85 (dd, J = 2.0, 9.0 Hz, 1H), 3.82 (s, 3H), 3.66 (s, 2H), 3.44 (s, 4H), 2.45 (s, 4H), 1.45 (s, 9H). LC-MS (ESI, *m/z*): Calcd for $C_{25}H_{30}BrN_3O_5S$ ([M+Na]$^+$) 526.2, found: 526.4. Compounds **5b**, **5c**, and **5d** were prepared with the same procedure according to **5a**, and the data were listed as below. *tert-Butyl 4-((5-methoxy-1-(phenylsulfonyl)-1H-indol-3-yl)methyl)piperazine-1-carboxylate (**5b**):* White solid (2.7 g, 90%), mp 59.8-61.2 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.87 (d, J = 9.0 Hz, 1H), 7.82 (d, J = 7.5 Hz, 2H), 7.49 (t, J = 7.5 Hz, 1H), 7.39 (t, J = 7.7 Hz, 3H), 7.13 (d, J = 2.4 Hz, 1H), 6.93 (dd, J = 2.4, 9.0 Hz, 1H), 3.80 (s, 3H), 3.56 (s, 2H), 3.39 (t, J = 4.3 Hz, 4H), 2.34 (t, J = 4.8 Hz, 4H), 9.05 (s, 9H); LC-MS (ESI, *m/z*): Calcd for $C_{25}H_{31}N_3O_5S$ ([M+H]$^+$) 486.2, found: 486.2. *tert-Butyl 4-((1-((4-isopropylphenyl)sulfonyl)-5-methoxy-1H-indol-3-yl)methyl)piperazine-1-carboxylate (**5c**):* White solid (3.9 g, 96%), mp 51.3-51.7 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.88 (d, J = 9.0 Hz, 1H), 7.74 (d, J = 12.2 Hz, 2H), 7.41 (s, 1H), 7.25 (t, J = 7.3 Hz, 2H), 7.13 (d, J = 2.4 Hz, 1H), 6.93 (dd, J = 2.4, 9.0 Hz, 1H), 3.81 (s, 3H), 3.57 (s, 2H), 3.39 (s, 4H), 2.90 - 2.85 (m, 1H), 2.36 (s, 4H), 1.45 (s, 9H), 1.18 (d, J = 7.0 Hz, 6H). LC-MS (ESI, *m/z*): Calcd for $C_{28}H_{37}N_3O_5S$ ([M+Na]$^+$) 550.2, found: 550.1. *tert-Butyl 4-((1-((4-fluorophenyl)sulfonyl)-5-methoxy-1H-indol-3-yl)methyl)piperazine-1-carboxylate (**5d**):* White solid (3.6 g, 93%), mp 138.0-139.3 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.84 (dd, J = 5.5, 8.9 Hz, 3H), 7.38 (s, 1H), 7.14 (d, J = 2.2 Hz, 1H), 7.07 (t, J = 8.4 Hz, 2H), 6.93 (dd, J = 2.2, 8.9 Hz, 1H), 3.81 (s, 3H), 3.57 (s, 2H), 3.40 (s, 4H), 2.36 (s, 4H), 1.45 (s, 9H). LC-MS (ESI, *m/z*): Calcd for $C_{25}H_{30}FN_3O5_S$ ([M+H]$^+$) 504.6, found: 504.6.

*(g). 1-((2-Bromophenyl)sulfonyl)-5-methoxy-3-(piperazin-1-ylmethyl)-1H-indole (**6a**):* To a solution of TFA, **5a** (1.0 g, 1.8 mmol) was added at RT under stirring, the reaction mixture was stirred at RT for 12 h. The resulted mixture was poured into water (10 mL), and then extracted with CH_2Cl_2 after the pH was adjusted to 7-8 with aqueous 5 N KOH solution, The combined organic layer was washed with water, brine, dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated under vacuum. The crude product was purified by silica gel column chromatography with CH_2Cl_2/CH_3OH (100:10) as eluent to afford **6a** as a pale yellow solid (0.8 g, 96%), mp 57.6-58.3 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.07 (d, J = 6.8 Hz, 1H), 7.64 (d, J = 10.0 Hz, 2H), 7.54 (d, J = 9.0 Hz, 1H), 7.45 (t, J = 7.5 Hz, 1H), 7.37 (t, J = 7.6 Hz, 1H), 7.18 (d, J = 2.0 Hz, 1H), 6.85 (dd, J = 2.0, 8.9 Hz, 1H), 3.81 (s, 3H), 3.63 (s, 2H), 2.98 (s, 4H), 2.55 (s, 4H). HRMS (ESI, *m/z*): Calcd for $C_{20}H_{22}BrN_3O_3S$ ([M+H]$^+$) 464.0638, found: 464.0639. Compounds **6b**, **6c**, and **6d** were prepared with the same procedure according to **6a**, and the data were listed as below. *5-Methoxy-1-(phenylsulfonyl)-3-(piperazin-1-ylmethyl)-1H-indole (**6b**):* White solid (0.7 g, 91%), mp 49.8-50.2 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.86 (d, J = 9.0 Hz, 1H), 7.83 (d, J = 7.5 Hz, 2H), 7.50 (t, J = 7.5 Hz, 1H), 7.40 (dd, J = 3.2, 7.6 Hz, 3H), 7.14 (d, J = 2.2 Hz, 1H), 6.92 (dd, J = 2.2, 9.0 Hz, 1H), 3.81 (s, 3H), 3.54 (s, 2H), 2.85 (s, 4H), 2.39 (s, 4H). HRMS (ESI, *m/z*): Calcd for $C_{20}H_{23}N_3O_3S$ ([M+H]$^+$) 386.1533, found: 386.1530. *1-((4-Isopropylphenyl)sulfonyl)-5-methoxy-3-(piperazin-1-ylmethyl)-1H-indole (**6c**):* Pale yellow solid (0.76 g, 94%), mp 50.2-51.8 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.87 (d, J = 9.0 Hz, 1H), 7.75 (d, J = 8.3 Hz, 2H), 7.42 (s, 1H), 7.23 (d, J = 8.3 Hz, 2H), 7.15 (d, J = 2.1 Hz, 1H), 6.93 (dd, J = 2.2, 9.0 Hz, 1H), 3.81 (s, 3H), 3.55 (s, 2H), 3.09 (s, 1H), 2.87 (s, 4H), 2.42 (s, 4H), 1.16 (d, J = 6.8 Hz, 6H). HRMS (ESI, *m/z*): Calcd for $C_{23}H_{29}N_3O_3S$ ([M+H]$^+$) 428.2002, found: 428.1999. *1-((4-Fluorophenyl)sulfonyl)-5-methoxy-3-(piperazin-1-ylmethyl)-1H-indole (**6d**):* White solid (0.72 g, 94 %), mp 56.5-58.3 ºC. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.85 (dd, J = 5.1, 8.9 Hz, 3H), 7.38 (s, 1H), 7.10 (d, J = 2.0 Hz, 1H), 7.08 (d, J = 8.4 Hz, 2H), 6.94 (dd, J = 2.0, 8.9 Hz, 1H), 3.81 (s, 3H), 3.59 (s, 2H), 3.02 (s, 4H), 2.56 (s, 4H). HRMS (ESI, *m/z*): Calcd for $C_{20}H_{22}FN_3O_3S$ ([M+H]+) 404.1432, found: 404.1439.

*(h). 1-[(2-Bromophenyl)sulfonyl]-5-[^{11}C]methoxy-3-[(4-methyl-1-piperazinyl)methyl]-1H-indole (O-[^{11}C]**2a**) and 1-[(2-bromophenyl)sulfonyl]-5-methoxy-3-[(4-[^{11}C]methyl-1-piperazinyl)methyl]-1H-indole (N-[^{11}C]**2a**), 5-[^{11}C]methoxy-3-((4-methylpiperazin-1-yl)methyl)-1-(phenylsulfonyl)-1H-indole (O-[^{11}C]**2b**) and 5-methoxy-3-((4-[^{11}C]methylpiperazin-1-yl)methyl)-1-(phenylsulfonyl)-1H-indole (N-[^{11}C]**2b**), 1-((4-isopropylphenyl)sulfonyl)-5-[^{11}C]methoxy-3-((4-methylpiperazin-1-yl)methyl)-1H-indole (O-[^{11}C]**2c**) and 1-((4-isopropylphenyl)sulfonyl)-5-methoxy-3-((4-[^{11}C]methylpiperazin-1-yl)methyl)-1H-indole (N-[^{11}C]**2c**), 1-((4-fluorophenyl)sulfonyl)-5-[^{11}C]methoxy-3-((4-methylpiperazin-1-yl)methyl)-1H-indole (O-[^{11}C]**2d**) and 1-((4-fluorophenyl)sulfonyl)-5-methoxy-3-((4-[^{11}C]methylpiperazin-1-yl)methyl)-1H-indole (N-[^{11}C]**2d**):* [^{11}C]CO_2 was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction in the small volume (9.5 cm^3) aluminum gas target provided with the Siemens RDS-111 Eclipse cyclotron. The target gas consisted of 1% oxygen in nitrogen purchased as a specialty gas from Praxair, Indianapolis, IN. Typical irradiations used for the development were 58 μA beam current

and 20 min on target. The production run produced approximately 37.0 GBq of $[^{11}C]CO_2$ at EOB. The precursor **3a-d** or **6a-d** (0.1-0.3 mg) was dissolved in CH_3CN (300 μL). To this solution was added aqueous KOH (2 M, 2 μL). The mixture was transferred to a small reaction vial. No-carrier-added (high molar activity) $[^{11}C]CH_3OTf$ that was produced by the gas-phase production method[33] within 12 min from $[^{11}C]CO_2$ through $[^{11}C]CH_4$ and $[^{11}C]CH_3Br$ with AgOTf column was passed into the reaction vial at RT until radioactivity reached a maximum (2 min), and then the reaction vial was isolated and heated at 80 °C for 3 min. The contents of the reaction vial were diluted with aqueous $NaHCO_3$ (0.1 M, 1 mL). The reaction vial was connected to a 3-mL HPLC injection loop. The labeled product mixture solution was injected onto the semi-preparative HPLC column for purification. The product fraction was collected in a recovery vial containing 30 mL water. The diluted tracer solution was then passed through a C-18 Light Sep-Pak cartridge, and washed with water (3 × 10 mL). The cartridge was eluted with EtOH (3 × 0.4 mL) to release the labeled product, followed by saline (10-11 mL). The eluted product was then sterile-filtered through a Millex-FG 0.2 μm membrane into a sterile vial. Total radioactivity was assayed and total volume (10-11 mL) was noted for tracer dose dispensing. The overall synthesis time including HPLC-SPE purification and reformulation was ~40 min from EOB. The decay corrected radiochemical yield was 40-50%. Retention times in the analytical HPLC system were: t_R **3a** = 5.23 min, t_R **6a** = 6.84 min, t_R **2a** = 7.44 min, t_R *O*-$[^{11}C]$**2a** = 7.51 min, t_R *N*-$[^{11}C]$**2a** = 7.49 min; t_R **3b** = 4.71 min, t_R **6b** = 6.21 min, t_R **2b** = 6.81 min, t_R *O*-$[^{11}C]$**2b** = 6.86 min, t_R *N*-$[^{11}C]$**2b** = 6.88 min; t_R **3c** = 6.76 min, t_R **6c** = 8.93 min, t_R **2c** = 9.46 min, t_R *O*-$[^{11}C]$**2c** = 9.58 min, t_R *N*-$[^{11}C]$**2c** = 9.61 min; and t_R **3d** = 5.03 min, t_R **6d** = 6.58 min, t_R **2d** = 7.12 min, t_R *O*-$[^{11}C]$**2d** = 7.19 min, t_R *N*-$[^{11}C]$**2d** = 7.23 min. Retention times in the preparative HPLC system were: t_R **3a** = 6.54 min, t_R **6a** = 7.25 min, t_R **2a** = 13.53 min, t_R *O*-$[^{11}C]$**2a** = 13.72 min, t_R *N*-$[^{11}C]$**2a** = 13.81 min; t_R **3b** = 6.32 min, t_R **6b** = 7.85 min, t_R **2b** = 12.01 min, t_R *O*-$[^{11}C]$**2b** = 12.27 min, t_R *N*-$[^{11}C]$**2b** = 12.23 min; t_R **3c** = 6.85 min, t_R **6c** = 9.59 min, t_R **2c** = 17.75 min, t_R *O*-$[^{11}C]$**2c** = 17.94 min, t_R *N*-$[^{11}C]$**2c** = 17.99 min; and t_R **3d** = 5.88 min, t_R **6d** = 7.24 min, t_R **2d** = 11.04 min, t_R *O*-$[^{11}C]$**2d** = 11.27 min, t_R *N*-$[^{11}C]$**2d** = 11.25 min.

Synthesis of carbon-11-labeled 5-HT$_6$R antagonists as new candidate PET radioligands for imaging of Alzheimer's disease

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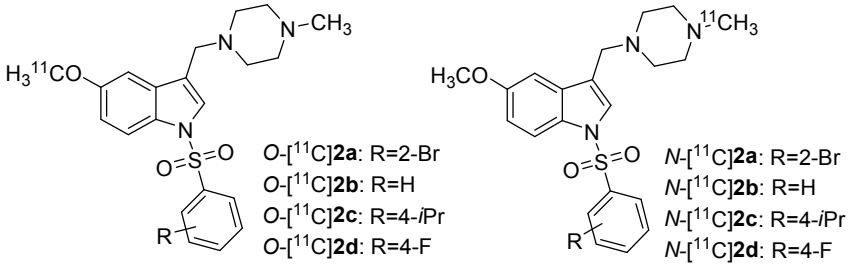

Carbon-11-labeled 5-HT$_6$R antagonists

Synthesis of carbon-11-labeled 5-HT_6R antagonists as new candidate PET radioligands for imaging of Alzheimer's disease

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- New carbon-11-labeled 5-HT_6R antagonists were synthesized.
- A fully automated multi-purpose [^{11}C]-radiosynthesis module was built up.
- A semi-preparative RP HPLC-SPE technique was employed in radiosynthesis.